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(11) **EP 2 302 438 A1**

(12) **EUROPEAN PATENT APPLICATION**

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(54) **Spherical aberration correction for an optical microscope using a moving infinity-conjugate relay**

(57)   An infinity-conjugate lens relay with a moving first lens is used to select a plane of interest from an image volume. This plane can be selected so that the image is corrected for spherical aberration due to non-ideal imaging conditions. This effectively will allow for deeper, corrected imaging for high power microscopes. Using an infinity-conjugate relay, this device has an ideal "bypass" mode for regular imaging without correction. The device also utilizes software that automatically controls the device for correcting live images.

Fig. 2

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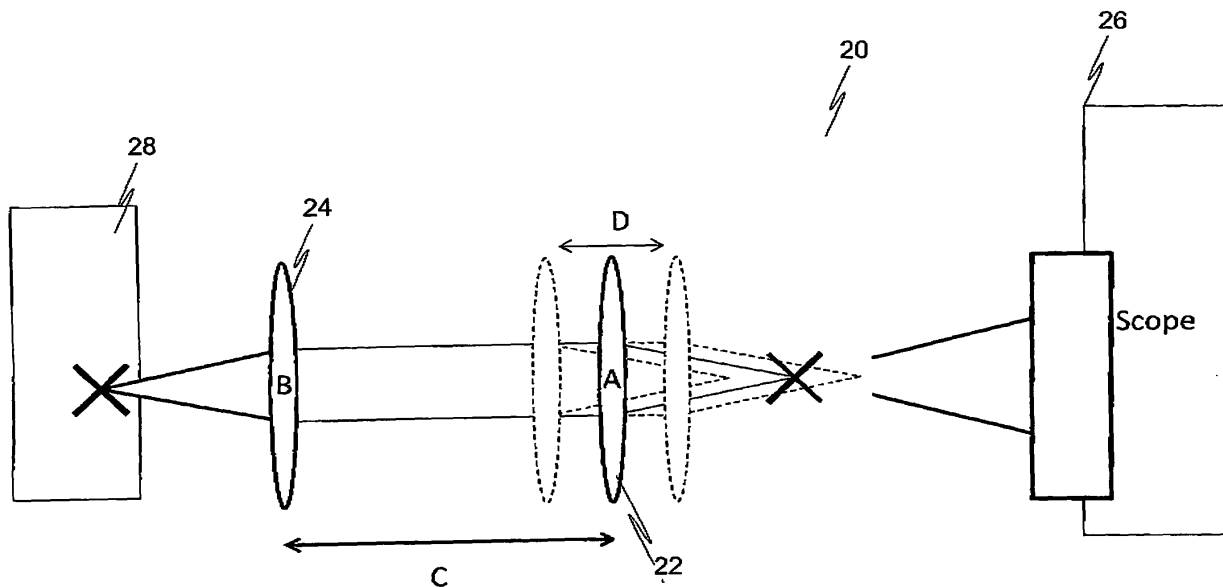
Description

RELATED APPLICATION DATA

[0001] This application claims the benefit of and priority under 35 U.S.C. §119(e) to U.S, Patent Application No. 61/244,561, filed September 22, 2009, entitled "Spherical Aberration Correction for an Optical Microscope using a Moving Infinity-Conjugate Relay," which is incorporated herein by reference in its entirety.

FIELD

[0002] An exemplary aspect of this invention generally relates to spherical aberration correction in optical microscopes. More specifically, an exemplary embodiment of this invention relates to a spherical aberration correction device. Even more specifically, an exemplary embodiment of the invention relates to a spherical aberration correction device using an infinity-conjugate relay. Even more specifically, an exemplary embodiment of the invention relates to an automated spherical aberration correction device.

BACKGROUND

[0003] High-power optical microscopes can use objectives with large numerical apertures (NA) resulting in high resolution imaging. In the case of oil or water immersion objectives these NAs can exceed 1.0. In any optical system, the first aberration that causes loss of signal and resolution is spherical aberration. Spherical aberration is the artifact in an imaging system caused by the inability of the optical system to focus axial and off-axis light from a point source to a single point. Modem objectives are highly corrected for spherical aberration, using multiple lens elements to eliminate the effect. However, these objectives are corrected only for a single ideal situation (e.g., for a specific cover glass thickness for samples on the cover glass). In practice, moving deeper into the sample introduces spherical aberration and is usually the limiting factor in deep imaging with these objectives. In addition, great variability in cover glass thickness from the manufacturer can cause significant losses of signal for imaging in what should be the ideal situation.

SUMMARY

[0004] A common optical microscope configuration when simplified comprises an infinity objective and a tube lens. A sample is imaged a specific distance from the objective and at the back focal length of the tube lens the magnified image is created. This image is then recorded by a camera or other imaging device. Most objectives are made so that points off the axis and in the focal plane are correctly relayed to the plane of the image. Points out of the focal plane (above or below) are also relayed to images before or after the measured image. These points, however, are not recorded because the imaging device only records a single plane. Effectively an "image volume" is created of the sample after the tube lens. It would be possible to capture three-dimensional data by moving the imaging device through the image volume.

[0005] Because points above and below the focal plane are no longer at the specified ideal location for the objective, spherical aberration will be introduced for these points. Consecutively worse spherical aberration will occur as the imaging device is moved away from the "ideal" image plane. Spherical aberration has a "direction" to it in that the point-spread-function (PSF) is distorted along the z-axis in one direction or another. Thus one can assign a positive and negative spherical aberration of varying degree.

[0006] When the sample being imaged is no longer at the specified ideal situation for the given objective, the image of the "ideal" focal plane will have spherical aberration. The true ideal focal plane (with no spherical aberration) will be located above or below the original plane. As the sample moves further from the ideal condition, the spherical aberration free image will move further from the original plane. For any of these conditions, one should be able to move the imaging device to the plane in the focal volume that represents the spherical aberration free focal plane. Thus by changing the location of the imaging device, one could correct for spherical aberration across a broad range of samples and imaging conditions. In most situations it is impractical to move the usually bulky imaging device, so a variable optical relay can be employed to change the plane of the image volume that is recorded.

[0007] Accordingly, one exemplary embodiment of the invention is directed toward a moving optical relay system for selecting a plane of interest from the focal volume. If this relay were to be motorized as well as the focus of the microscope, one could in an automated fashion correct for the spherical aberration in the current sample plane. Typically this would involve software to select the position of the relay based on the quality of the image.

[0008] Primarily, there are two sources of spherical aberration in a given sample. The first is due to the conditions of the sample-usually variations in the thickness of the cover glass. This source of aberration is usually invariant over the sample, but is very hard to predict. To correct for this, the sample must be directly measured and the best plane of interest of the image volume chosen based on the measurement of the sample at several planes. The second source of aberration is due to imaging deeper into the sample. The spherical aberration varies with depth for any given sample medium for any given objective in a known manner. An algorithm can be developed that will give the plane of the image volume needed for any given depth of the sample. The end result is that one has a curve that represents the change in plane location vs. sample depth and a random offset to this curve caused by the sample. The offset can be found empirically and then any depth in the sample can be corrected using the calculated curve.

[0009] One exemplary variable relay system involves two positive lenses. The first lens is placed the distance of its focal length from the image plane. By moving this lens, the desired image plane can be selected. The second lens is placed its focal length from the detector. The image plane that is the focal length of the first lens away will be relayed at infinity to the second lens which images it onto the detector. In this way, the detector can be fixed in its location, while only one lens element needs to be moved Ideally the distance between the two lenses is equal to the sum of their focal lengths, As the first lens moves, this ideal situation will change, with the primary consequence being that the second lens must be of greater diameter. If positioned correctly and if the two lenses are equal, a "zero" point can be established where the original focal plane is imaged with no additional magnification or distortion.

[0010] In accordance with another exemplary embodiment, one could use a fast, accurate linear motion device to move the first lens. This would allow the application where the position of the lens is continuously varied as the imaging condition varies (usually by moving deeper in the sample). For example this could allow three-dimensional imaging where each plane in z is individually corrected for spherical aberration. In practice such a device would allow for deeper imaging into a sample.

[0011] If the linear motion device were fast enough to perform the required motion in under the transfer time of the detector (for many scientific cameras this time is near 10ms), there would be no performance loss while correcting the spherical aberration for each image.

[0012] According to one exemplary embodiment, the linear motion device is a moving coil actuator which can provide the speed and precision necessary.

[0013] The exemplary apparatus can comprise a two lens relay where the first lens can move relative to the other and a means, such as a motor for moving the first lens.

[0014] This apparatus when combined with an optical microscope and an imaging device would provide a way to correct for spherical aberration in an automated fashion.

[0015] This device has a first exemplary advantage that when it is in the "zero" position it effectively has no effect on the image-as if the device were not present. This allows the microscope to be used in a normal fashion when spherical aberration correction is not desired or needed. Because this device can also be made with fast motion control, it will allow for spherical aberration correction without affecting the system performance.

[0016] Aspects of the invention are thus directed toward spherical aberration correction in optical microscopes.

[0017] Aspects of the invention are also directed toward automatic spherical aberration correction in optical microscopes.

[0018] Still further aspects of the invention are directed toward a spherical aberration correction device.

[0019] Even further aspects of the invention are directed toward a spherical aberration correction device using an infinity-conjugate relay.

[0020] Still further aspects of the invention are directed toward a motorized relay lens system for selecting a plane from an image volume.

[0021] Even further aspects of the invention are directed toward a fast linear motion device such that the desired motion can happen in less than the frame transfer time of a scientific camera.

[0022] Even further aspects of the invention are directed toward automated control and software for the device.

[0023] Still further aspects of the invention relate to an apparatus for a spherical aberration correction system including an infinity-conjugate relay system with two lenses, means for moving the first lens along the optical axis, means for controlling the motion of the first lens to select the desired image plane and means for controlling the motion of the first lens while imaging.

[0024] The aspect above, where the first lens is motorized.

[0025] Any of the above aspects, where the first lens is motorized by using a moving coil actuator.

[0026] Any of the above aspects, where the first lens is motorized using a stepper-motor.

[0027] Any of the above aspects, where the first lens is moved using a manual focusing device.

[0028] Any of the above aspects, where the motorization control device is synchronized with the detector.

[0029] Any of the above aspects, where the means for moving the first lens can do so in under the transfer time of the imaging camera.

[0030] Any of the above aspects, were instead of an imaging relay device, the detector itself is moved to select the desired image plane.

[0031] Any of the above aspects, where the apparatus is combined with an optical microscope.

[0032] Any of the above aspects, where the apparatus is combined with an electronic imaging device such as a camera.

[0033] Any of the above aspects, where the apparatus is combined with a scanning microscope.

[0034] Any of the above aspects, where the scanning microscope is a confocal microscope.

[0035] Any of the above aspects, where the scanning microscope is a two-photon microscope.

[0036] Any of the above aspects, where the apparatus has a "zero" mode where the effective image is unaltered from the image were the apparatus not present.

[0037] Any of the above aspects, where the apparatus is automated and controlled with a computer program.

[0038] Any of the above aspects ,where the computer program uses a calculated curve to determine the position of the first lens for a given sample depth.

[0039] These and other features and advantages of this invention are described and, or are apparent from, the following detailed description of the exemplary embodiment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The exemplary embodiments of the invention will be described in detail, with reference to the following figures wherein:

[0041] Figure 1 illustrates the basic concept of the focal volume and how it relates to spherical aberration.

[0042] Figure 2 illustrates an exemplary lens system using an infinity conjugate relay with a moving front lens as a spherical aberration correction device.

[0043] Figure 3 illustrates how spherical aberration can be corrected by selection of an appropriate plane in the image volume.

[0044] Figure 4 illustrates an exemplary software and hardware control flow for finding the desired plane in the image volume.

[0045] Figure 5 illustrates an exemplary software and hardware control flow for automatically correcting for spherical aberration during live imaging.

[0046] Figure 6 illustrates an environmental perspective view of an exemplary embodiment of a spherical aberration correction device.

DETAILED DESCRIPTION

[0047] The exemplary embodiments of this invention will be described in relation to microscopes, imaging systems, and associated components. However, it should be appreciated that, in general, known components will not be described in detail. For purposes of explanation, numerous details ate set forth in order to provide a thorough understanding of the present invention. It should be appreciated however that the present invention may be practiced in a variety of ways beyond the specific details set forth herein.

[0048] Figure 1 illustrates the concept of a focal volume as formed by an infinity objective. It also shows how spherical aberration is related to the focal volume. Inside a typical microscope there is an objective A which relays images at infinity to a tube lens B. If this is an oil objective, then typically there would be an interface C between the oil and the sample which is often aqueous in biological research - Typically this is a cover glass. When the sample of interest is on this interface, then the objective's ideal conditions are met-at the imaged plane D of the focal volume E, there is no spherical aberration. There is positive and negative spherical aberration before and behind this plane When imaging deeper into the sample, ideal conditions are not met, and the imaged plane F of the focal volume is no longer aberration free. There exists, however a plane where there is no spherical aberration before or after the imaged plane.

[0049] Figure 2 illustrates an exemplary optical system 20 according to this invention. The optics consists of a front lens A (22) and a back lens B (24) that are placed between the microscope 26 and imaging device (e.g., camera 28) such that they form an infinity-conjugate relay. Ideally, the distance between the lenses C is the sum of the focal lengths of the lenses. This forms a simple relay of the image from the tube lens onto the imaging device, that is, there is no introduced change into the image as if the device were not present. The front lens can move distance D, allowing selection of a specific plane within the focal volume.

[0050] Figure 3 illustrates the process of correcting spherical aberration. When the sample is at the ideal location A, the imaged plane is aberration free. When imaging in a non-ideal situation B, the imaged plane is no longer aberration free. The imaged plane is changed C to the plane that is aberration free using the device according to this invention. The focus D is changed to refocus on the sample of interest, but now with no aberration.

[0051] Figure 4 illustrates the software and hardware control for finding the plane in the focal volume that is least aberrated. The digital image of the sample of interest 40 is put through an algorithm 42 which generates a fitness number such that a higher fitness number represents a less aberrated image. The position of the spherical aberration correction device is then changed in control process 44 that can be realized by, for example, a control module (not shown), This causes the sample of interest to be no longer in focus. The focus of the microscope is then changed until the sample is back into focus in process 46. Another digital image is then taken and the process is repeated, A curve for fitness vs. spherical aberration correction device position 48 is then generated along with the curve for z position vs. device position 49. The correct position of the spherical aberration correction device is the position when the curve of fitness 48 has a maximum,

[0052] Figure 5 illustrates the software and hardware control for automatically correcting the spherical aberration during imaging. The imaging device 50 reports electronically that it is done with the current image. While the image is being transferred, the microscope 52 performs any required automation such as changing the z position of the objective. The microscope then reports the currant z position to, for example a controller, such as a computer-based controller, for example a synchronization module. From the data acquired previously, the correct spherical aberration device position and the corresponding change to the microscope focus can be calculated by process 54. The device is then moved in step 56 before the next image is begun.

[0053] Figure 6 illustrates an exemplary embodiment of a spherical aberration correction device 62. The top panel 62 can optionally be made transparent so the insides can be seen. The movable front lens is located on a fast linear stage 64 with associated motor and control circuit (not shown). The back lens 66 is located near the output of the device.

[0054] The exemplary techniques illustrated herein are not limited to the specifically illustrated embodiments but can also be utilized with the other exemplary embodiments and each described feature is individually and sep-

arately claimable.

[0055] The systems of this invention also can cooperate and interface with a special purpose computer, a programed microprocessor or microcontroller and peripheral integrated circuit element(s), an ASIC or other integrated circuit, a digital signal processor, a hard-wired electronic or logic circuit such as discrete element circuit, a programmable logic device such as PLD, PLA, FPGA, PAL, any comparable means, or the like. The term module as used herein can refer to any known or later developed hardware, software, firmware, or combination thereof, that is capable of performing the functionality associated with that element. The terms determine, calculate, and compute and variations thereof, as used herein are used interchangeable and include any type of methodology, process, technique, mathematical operational or protocol.

[0056] Furthermore, the disclosed system may use control methods and graphical user interfaces that may be readily implemented in software using object or object-oriented software development environments that provide portable source code that can be used on a variety of computer or workstation platforms that include a processor and memory. Alternatively, the disclosed control methods may be implemented partially or fully in hardware using standard logic circuits or VLSI design. Whether software or hardware is used to implement the systems in accordance with this invention is dependent on the speed and/or efficiency requirements of the system, the particular function, and the particular software or hardware systems or microprocessor or microcomputer systems being utilized.

[0057] It is therefore apparent that there has been provided, in accordance with the present invention a spherical aberration correction device. While this invention has been described in conjunction with a number of embodiments, it is evident that many alternatives, modifications and variations would be or are apparent to those of ordinary skill in the applicable arts. Accordingly, it is intended to embrace all such alternatives, modifications, equivalents and variations that are within the spirit and scope of this invention.

Claims

1. A spherical aberration correction device comprising:

    an imaging relay system that selects a desired plane from an image volume and relays that plane to an imaging device.

2. The device of claim 1, further comprising of a software system to automatically adjust the device to correct for spherical aberration.

3. The device of claim 2, wherein the software system is adapted to adjust for spherical aberration based

only on image data.

4. The device of claim 2 or 3, wherein the software system is adapted to use a stored equation or numerical formula to adjust the spherical aberration correction for any given sample depth.

5. The device of claim 4, wherein the software system is adapted to automatically adjust the correction during live imaging.

6. The device of claim 5, wherein the software also uses an empirically derived offset to the correction to account for the sample conditions.

7. The device according to any one of the preceding claims, wherein spherical aberration is corrected for each plane individually in a three-dimensional data set.

8. A spherical aberration correction device, preferably according to any one of the preceding claims, comprising:

    means for an imaging relay system to select a desired plane from an image volume; and
    means for relaying that plane to an imaging device.

9. The device according to any one of the preceding claims, further comprising an infinity conjugate relay system.

10. The device according to any one of the preceding claims, further comprising a motorized first lens.

11. The device of claim 10, wherein a moving coil actuator is used to move the first lens.

12. The device according to any one of the preceding claims, further comprising a motion control system that controls focus of a microscope,

13. The device of claim 10 or 11, further comprising a synchronization module that synchronizes the imaging device and the motion control system.

14. The device of claim 10, 11 or 13, wherein motion can occur during a transfer time of the imaging device,

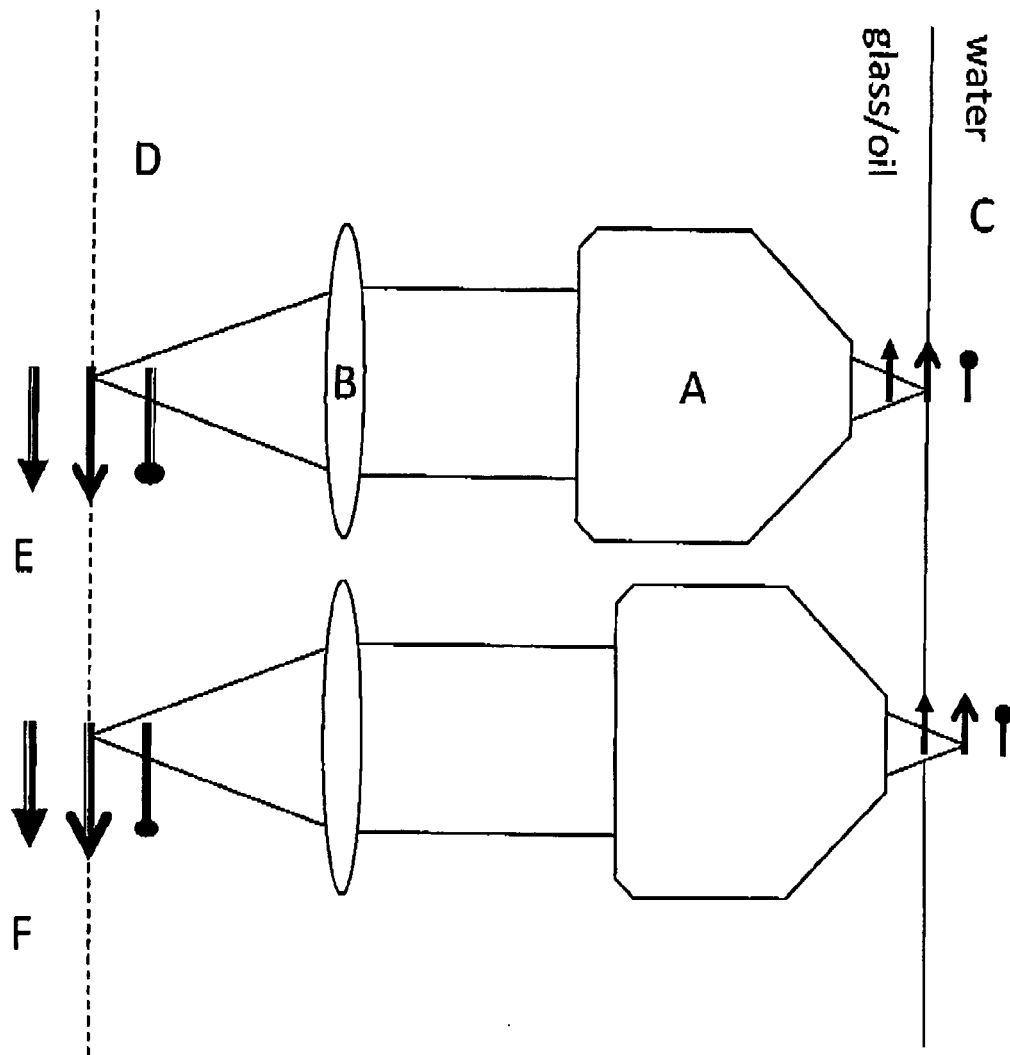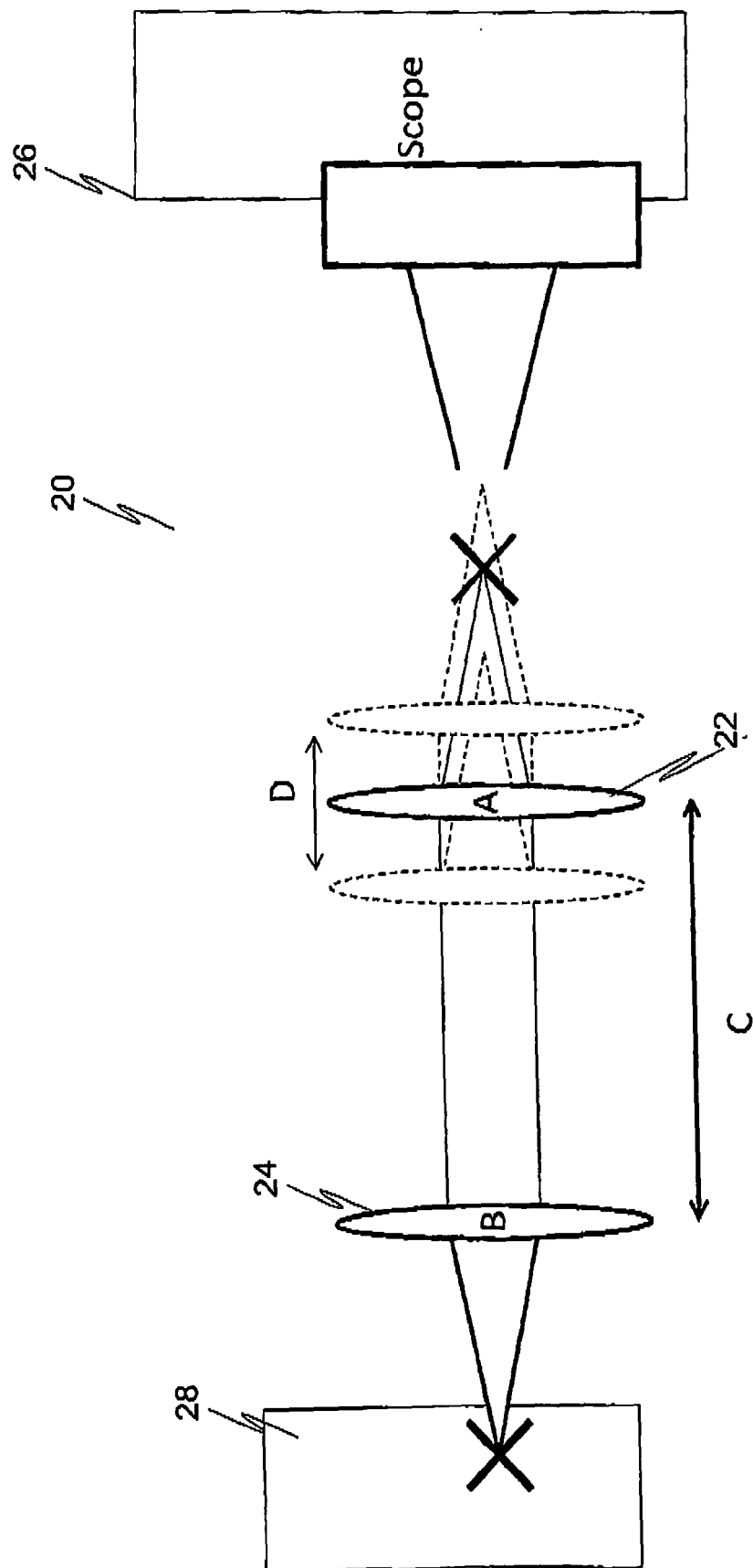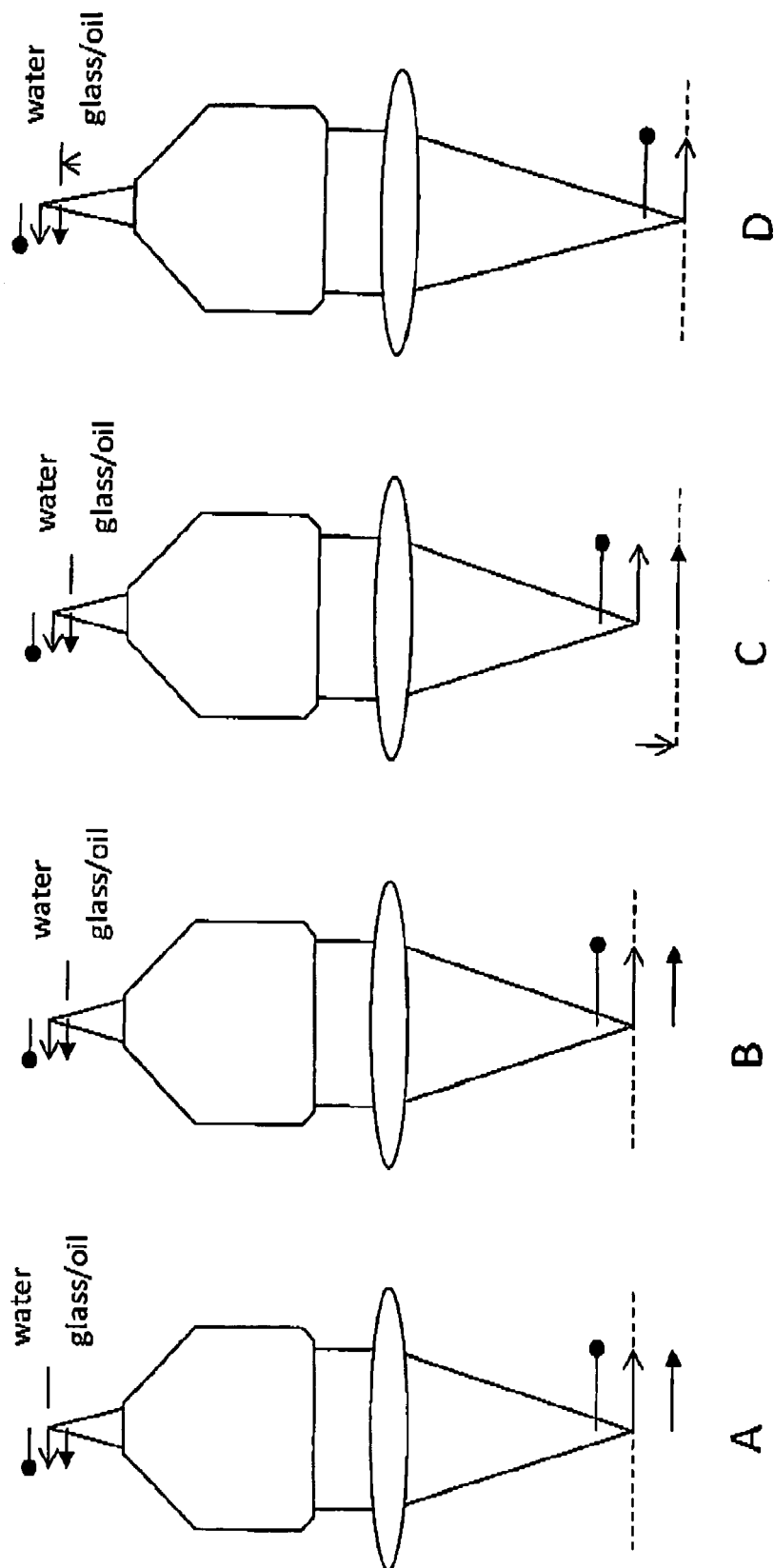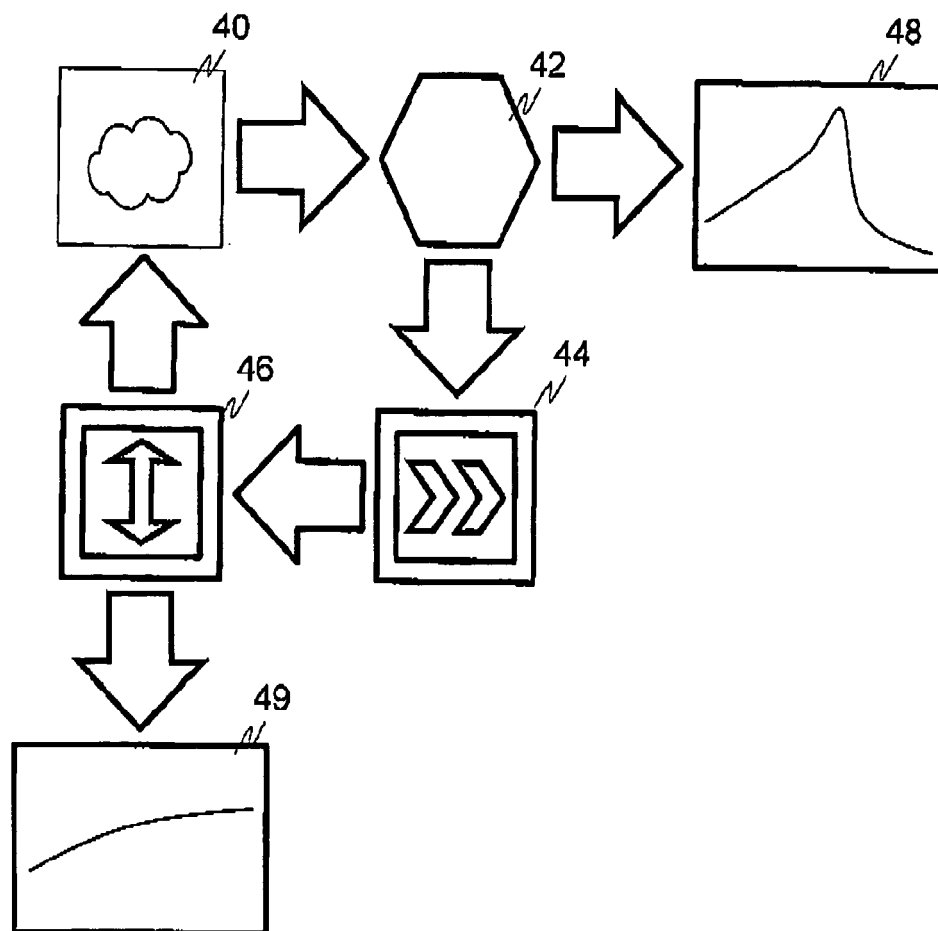15. The device of claim 10, 11, 13 or 14, wherein the motion can occur during imaging.

Fig. 1

Scope

26

20

22

D

A

24

B

C

28

Fig. 2

Fig. 3

Fig. 4

Fig. 5

66

60

62

64

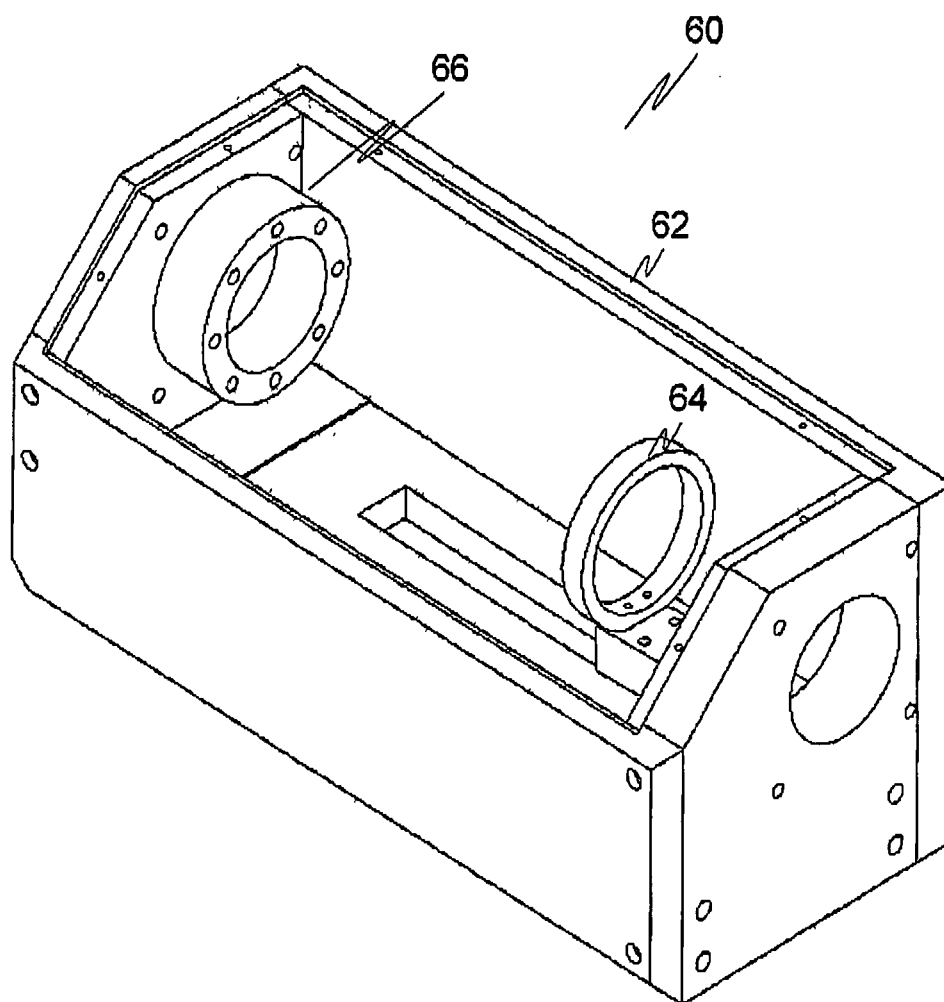Fig. 6

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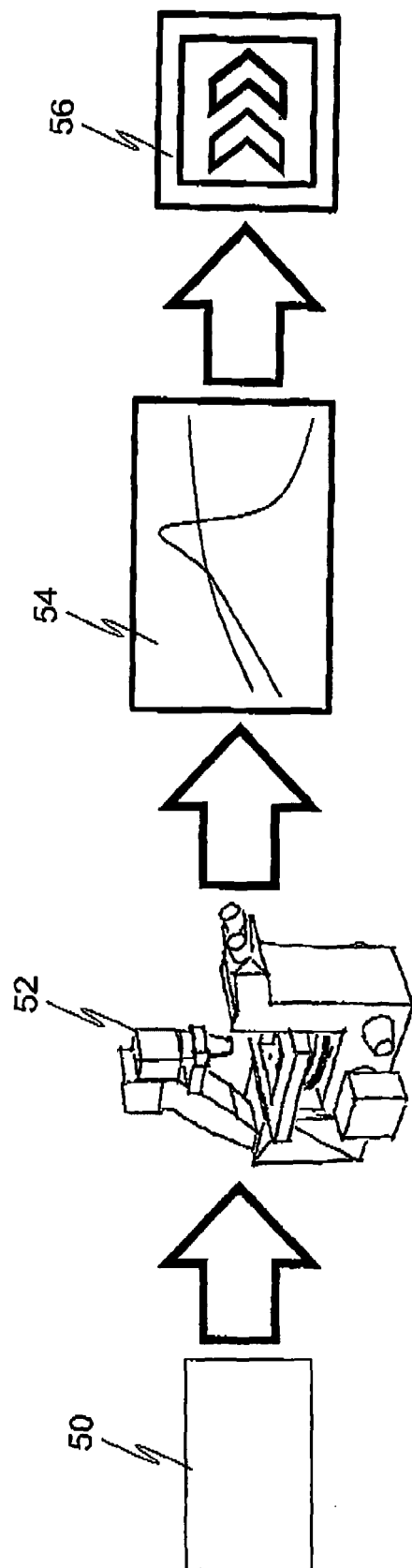
EUROPEAN SEARCH REPORT

Application Number

EP 10 01 0141

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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			TECHNICAL FIELDS SEARCHED (IPC)
			G02B G11B

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	15 February 2011	Ward, Seamus

EPO FORM 1503 03.82 (P04C01)

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ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
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